Barley DNA Extraction in Microcentrifuge Tubes

Materials in PrecisionPak[™]

- Navy or PC2 Bead Lysis Kit
- FoamBlocker
- Plant DNA Extraction Kit



Supplied in the Kit:

Plant DNA Extraction Kit Contents	
Solution LA (Lysing)	60 mL
Solution PA (Precipitation)	15 mL
Solution CA (Clean Up)	30 mL

Methods

Homogenization

- 1. Add 500 µL of Solution LA into each lysis kit tube.
- 2. Add $1-2 \text{ cm}^2$ or 50 mg of plant material to the buffer-filled tubes. *Note:* Cut sample into small pieces – larger sample size may affect complete homogenization with the recommended homogenization setting.
- 3. Set the <u>Bullet Blender</u> at speed 12, time 3 minutes and homogenize the samples. If using other homogenizer models, refer to the manufacturer's instructions for settings.
- 4. Remove the tubes and visually inspect the samples to confirm complete homogenization (Figure 1). Note: If unhomogenized tissue is seen, homogenize for additional 30 seconds.

To Be Supplied by User:

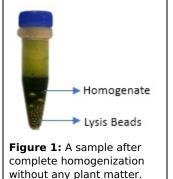
Molecular Grade Water

Precipitation

- 1. Remove approximately 500 μ L of the homogenate from the lysis tubes and transfer to a new tube containing an equal amount of Solution LA.
- 2. Add 100 µL of Solution PA to each tube and mix thoroughly by inversion.
- 3. Centrifuge the samples at 10,000 RPM for 5 minutes to pellet the contaminants.

Cleaning

- 1. Transfer 750 μ L of the supernatant that contains the DNA into new tubes with 750 μ L of Solution CA. Mix thoroughly by inversion and incubate at room temperature for 5 minutes.
- 2. Centrifuge the solutions at 13,000 RMP for 7 minutes to pellet the DNA.
- 3. Remove the supernatant with a 1 mL pipette.







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- 4. Re-spin the tubes briefly and remove any remaining supernatant with a 200 μL pipette.
- 5. Add 30 μ L of molecular grade water to the pellet.
- 6. Allow the DNA to rehydrate for 10 minutes (about 20% rehydration) or overnight (100% rehydration).
- 7. Analyze quality (OD₂₆₀/OD₂₈₀) and yield using a NanoDrop[™] or other spectrophotometer and agarose gel (Data of gDNA is shown in Figure 2 and Table 1).
- 8. Isolated DNA can be stored at 4°C for up to a week or at -20°C for long term storage.



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